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Morphological changes of the peritoneal membrane in patients with long-term dialysis

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Abstract

Morphological alterations of peritoneum in chronically dialyzed patients involve fibrosis and angiogenesis as pathogenic mechanisms. The aim of this retrospective study was to evaluate morphological changes of peritoneum in chronic peritoneal dialysis (PD) at 4, 8, 12, and 14 years. Peritoneal changes were investigated in 110 patients with end stage renal failure, which were included in a PD program. Intraoperative biopsies were grouped in four study Groups (A: 1–48 months, B: 49–96 months, C: 97–144 months, and D: 145–168 months), and were processed histologically and stereologically. Mesothelial denudation was found in percentage volumes of 5.49% – Group A, 16.10% – Group B, 16.68% – Group C and 19.88% – Group D. Reduplication of the basement membrane was observed in patients with over five years of PD. Interstitial stromal fibrosis recorded percentage volumes of 25.49% (Group A), 26.10% (Group B), 35.85% (Group C) and 56.63% for the patient with 14 years of PD. Subendothelial hyalinizing vasculopathy was recorded in percentage volumes of 2.22%, 6.63%, 9.16% up to 9.20%. Vascular permeability reduction was recorded as decreasing percentage volumes from 22.59% to 12.81%, 7.77% and 7.37%. Perivascular inflammation was marked in the serosa of the patients in Group A (4.55%). Calcifications recorded percentage volumes of 1.63% at eight years, 3.74% at 12 years and 4.03% at 14 years of PD. Peritoneal morphological changes appear at 3–4 years of PD and progressively aggravate with long-term PD.

Keywords: submesothelial fibrosis, neoangiogenesis, inflammation, vasculopathy, peritoneal dialysis.

Introduction

The peritoneal serosa behaves as a composite anatomical and functional membrane [1]. In normal subjects, it consists of a monolayer of mesothelial cells on a basement membrane and a layer of connective tissue embedding cells, blood vessels, and lymphatics [2]. The peritoneal mesothelium is composed by a single polygonal cell layer with apical microvili, which are widening the transperitoneal exchange surface [3]. In inflammation, the fibrinolytic activity of the peritoneal mesothelium is reduced while procoagulating activity is enhanced; thus, a fibrin pellicle is formed on the mesothelial surface. The mesothelial basement membrane includes collagen fibers and acts as a selective barrier for cells; it is also involved in mesothelial cells regeneration [4]. The capillary endothelium can include pores among neighbor endothelial cells (Figure 1). As referred to peritoneal dialysis (PD), the vascular endothelium corresponds to the blood compartment, in the dialyser and to the lymphatic compartment. Capillary and postcapillary permeability is controlled by the endothelium.

Peritoneum functions as a semipermeable imperfect and selective dialysis membrane for water and solutes between endoperitoneal and blood compartments and its usage as support for long-term PD are progressively impaired by the association of three elements: (1) chronic placement of the dialysis catheter; (2) usage of hypertonic incompatible dialyzing solutions; (3) severe acute or recurrent episodes of infectious peritonitis.

Peritoneal dialysis is used for the treatment of chronic renal failure. Morphological changes of peritoneum due to the process of long-term dialysis involve the mesothelium, the intercellular junctions, the interstitial tissue and blood vessels [5]. Morphological changes of peritoneum are also found in complications of chronic PD [5]. In such situations, the permeability of peritoneum is modified and the lymphatic drainage is enhanced [5]. PD can modify peritoneal morphology and structure and the progressive alterations may lead to peritoneal failure [2]. On other hand, the morphological alterations of peritoneum impair the accuracy of PD [6–10]. It was thus aimed at studying retrospectively the morphological changes of peritoneum in chronic PD patients (at 4, 8, 12, and 14 years of treatment).

Patients and Methods

The present retrospective study assessed the morphological changes of peritoneum in 110 patients (58 females/ 52.72% and 52 males/47.27%) with terminal renal chronic failure enrolled for PD procedures (lasting from one to 168 months) in the Department of Nephrology of "Dr. C. I. Parhon" Hospital, Iassy, Romania. Patients were hospitalized in the II[nd] Surgical Clinic of "Sf. Spiridon" Hospital, Iassy, between 1.01.2003–31.12.2010 for catheter

removal due to various causes: (1) abdominal parietal sepsis on the output catheter site (recurrent episode, with/without infectious peritonitis) in eight (7.27%) patients, five females and three males; (2) severe iterative microbial peritonitis, non-responsive to conservative treatment, in 46 (41.81%) patients, 25 females and 21 males; (3) dialysis inefficiency, in 26 (23.6%) patients, 14 females and 12 males; (4) association of the two later-discussed pathologies, in 10 (9%) patients, six females and four males; (5) patient request, in six (5.45%) cases, two females and four males; (6) prior to renal transplantation, in eight (7.27%) patients, two females and six males; (7) bowel occlusion/subocclusion by adherential pathology, in six (5.45%) patients, four females and two males.

Surgical exploration of the peritoneal cavity performed a macroscopic evaluation of the mesothelial serosa and of peritoneal filling, if present.

Intraoperative biopsies were sampled usually from the antero-lateral parietal peritoneum (8–12×8–12 mm sized samples); there were also samples harvested from the visceral peritoneum (segmentary enterectomy samples), fibrous enterolysis samples and samples from the resected viscero-parietal bridles. Samples were divided, according to PD duration, in four study groups and assessed (Table 1).

Table 1 – ***Peritoneal biopsies distribution in patients with terminal stage renal failure according to peritoneal dialysis duration***

Group	PD duration [months]	No. of patients			%		
		Total	*F*	*M*	*Total*	*F*	*M*
A	1–48	92	48	44	83.63	43.63	40.00
B	49–96	12	7	5	10.90	6.36	4.55
C	97–144	5	2	3	4.54	1.81	2.72
D	145–168	1	1	–	0.90	0.90	–

PD – Peritoneal dialysis; F – Females; M – Males.

From the 290 episodes of infectious peritonitis in the patients from Department of Nephrology involved in the PD program (for the considered duration), 46 required surgery. Maximal number of peritoneal infectious episodes, correlated with a functional PD catheter ranged between 5–11/patient in Group A, 9/patient in Group B, 6/patient in Group C and none in the last group (ultrafiltration impairment – UF).

Peritoneal and fibrous samples were paraffin embedded, cut and stained for histological examination (Hematoxylin–Eosin, trichromic Szekely, Gordon–Sweet and Van Gieson stains). Biopsy samples were histologically stereologically assessed to emphasize mesothelial cells integrity, the presence of denudation areas, submesothelial interstitial changes, the presence of acute or chronic inflammation, vascular abnormalities generated by subendothelial hyaline sclerosis in post-capillary venulae and arteriolae.

Stereological assessment

Stereological interpretation for quantitative measurements was performed on representative microscopic samples from biopsies in the four groups, using a 40× objective. Images were recorded by an image acquisition system and interpreted by Prodit 5.2 image analysis software. Thus, we were able to perform specific measurements by selecting an automated method.

Stereology option was used to evaluate percentage volumes (%) for structural elements in the peritoneal samples (mesothelium, connective tissue, blood vessel lumen, inflammatory lesion) in patients with different PD duration (at four years/48 months interval) by comparison with normal peritoneum. This method combines test-dots and test-lines in a standard surface, allowing the calculation of the selected percentage volumes.

Digital superposition of a standard grid over the acquired histological image is performed. The grid was adapted by the observer to the specific tissue parameters, in order to produce optimal quantification conditions.

A 40× objective together with the Weibel parallels test grid was used; the distance between two points was of 19.39 μm. For each sample, the test grid was displaced by the observer starting from mesothelium toward lower limit, and then reversely in the near area until reaching a test surface of 528 points.

Statistical evaluation reported percentage volumes for reference elements in each sample, then on study groups, while statistically important data were depicted in specific diagrams.

Results

At surgical inspection of the peritoneal cavity in patients with infectious peritonitis, peritoneal fluid was purulent in 50% cases. Parietal and visceral peritoneum in patients with PD over five years showed a tanned aspect with hyperpigmented areas (of 1–2 cm) and hard-bound on palpation (Figure 2). Proximal to distal investigation of the peritoneal barrier (from the interface of peritoneal areas artificially induced by PD toward endocapillary space) showed the following changes, according to histological and stereological results: (1) mesothelial denudation was present in a percentage volume of 5.49% for patients in Group A, 16.10% in Group B, 16.68% in Group C, and 19.88% in Group D (Figures 3–5); (2) reduplication of the basement membranes (evaluated histologically) was recorded on the 11 patient samples, which undergone over five years (60 months) of PD (61.11%); (3) submesothelial interstitial stromal fibrosis was quantitatively evaluated in progressive percentage volumes, from 25.49% in Group A to 26.10% in Group B, 35.85% in Group C, and 56.63% in the patient with 14 years of PD (Figures 6–8); (4) fibrin presence was noticed in percentage volumes of 3.03% (Group A), 26.22% (Group B), 29.77% (Group C), and 30.06% (Group D) (Figure 9); (5) hyalinizing vasculopathy by subendothelial hyaline deposits was observed in increasing percentage volumes from 2.22% to 6.63%, 9.16%, and 9.20% (Figure 10); (6) together with progressive subendothelial vascular hyalinosclerosis, it was noticed a vascular lumen distortion with different stenosis degrees up to complete vascular obliteration. Thus, vascular permeability was progressively reduced in percentage volumes from 22.59% in Group A to 12.81% in Group B, 7.77% in Group C, and 7.37% regarding the peritoneum with 14 years of PD exposure (Figure 11); (7) perivascular inflammatory process was more important in patients in the Group A (4.55%), with progressively decreasing percentage volumes, consistent with PD duration, of 2.98%, 1.87% and 1.33% in the remaining patient groups; (8) calcification occur-

rence (mainly in the hyalinized vessel walls) was observed following five years of PD (with percentage volumes of 1.63% up to eight years, 3.74% up to 12 years and 4.03% at 14 years of PD) (Table 2, Figure 12).

Table 2 – *Evaluation of the percentage volumes regarding morphological peritoneal changes*

Group	PD duration [years]	Lesion type (percentage volume)						
		Mesothelial denudation	*Submesothelial stromal fibrosis*	*Fibrin deposits*	*Subendothelial vascular hyalinosis*	*Vascular permeability*	*Perivascular inflammation*	*Calcification*
A	0–4	5.49	25.49	3.03	2.22	22.59	4.55	–
B	5–8	16.10	26.10	26.22	6.63	12.81	2.98	1.63
C	9–12	16.68	35.85	29.77	9.16	7.77	1.87	3.74
D	13–14	19.89	56.63	30.06	9.20	7.37	1.33	4.03

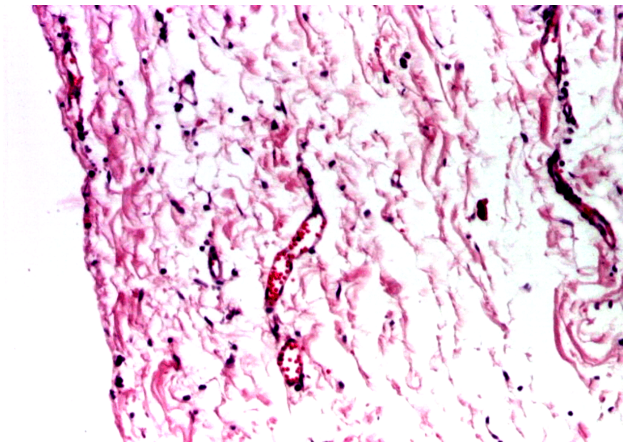

Figure 1 – *Normal peritoneum (with a single layer of mesothelial cells, stromal and permissive vascular compartments). HE staining, ×100.*

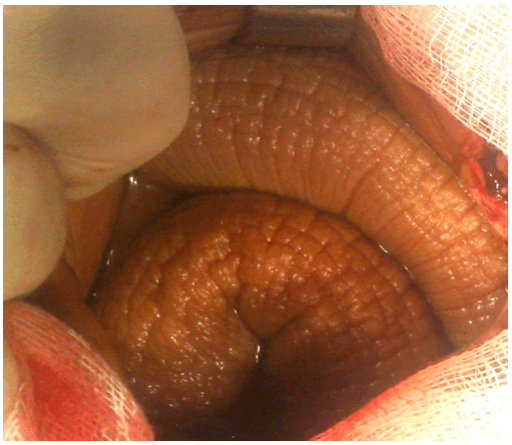

Figure 2 – *Tanned and hardbound peritoneum. Macroscopic aspect.*

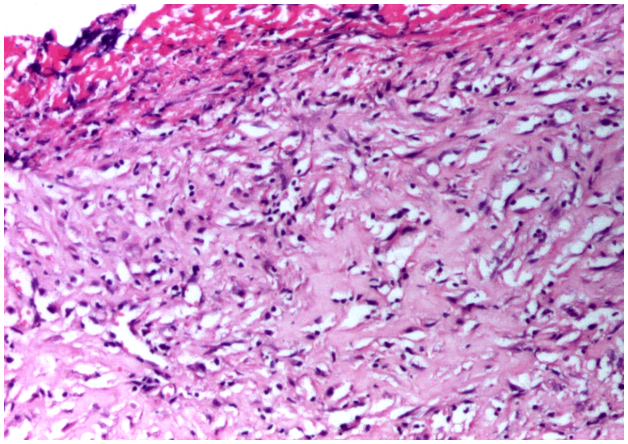

Figure3 – *Mesothelial denudation, fibrin deposits and stromal inflammatory infiltrate (five years of PD). HE staining, ×100.*

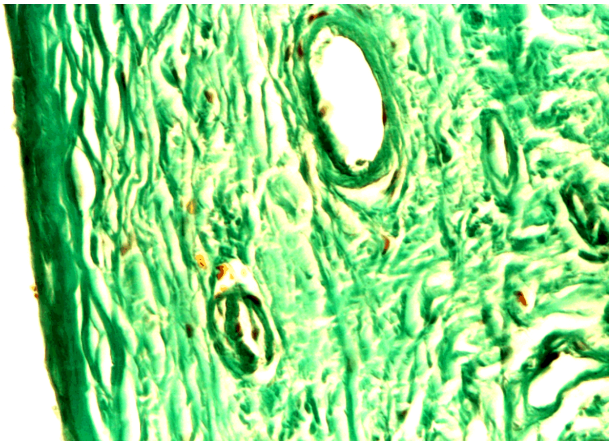

Figure 4 – *Fibrous mesothelial exfoliation, starting subendothelial hyalinization (four years of PD). Trichromic Szekely staining, ×200.*

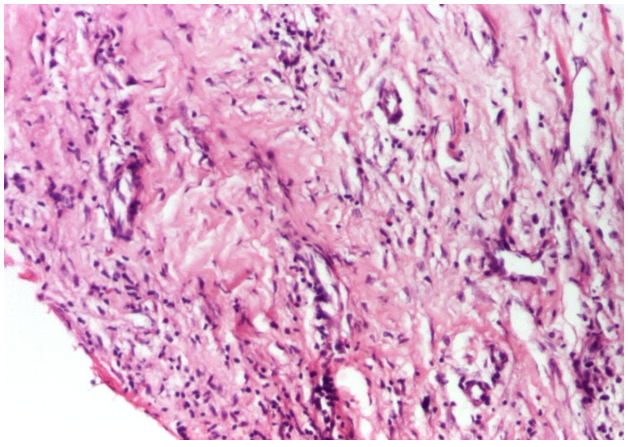

Figure 5 – *Infectious peritonitis (discontinuous mesothelial layer and diffuse polymorphous inflammatory infiltrate, at three years of PD). HE staining, ×100.*

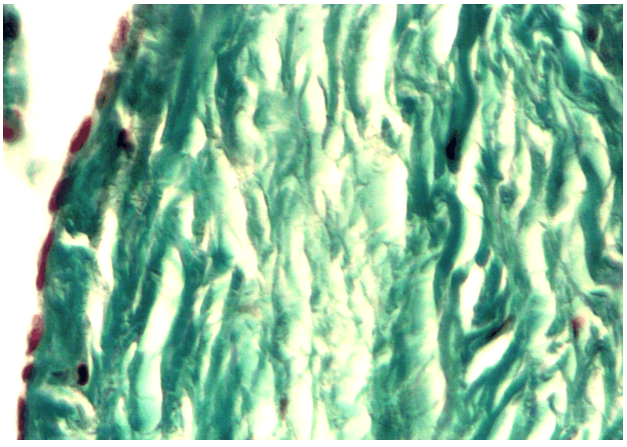

Figure 6 – *Peritoneum with a continuous layer of mesothelial cells and subsequent "band" fibrosis (at four years of PD). Trichromic Szekely staining, ×400.*

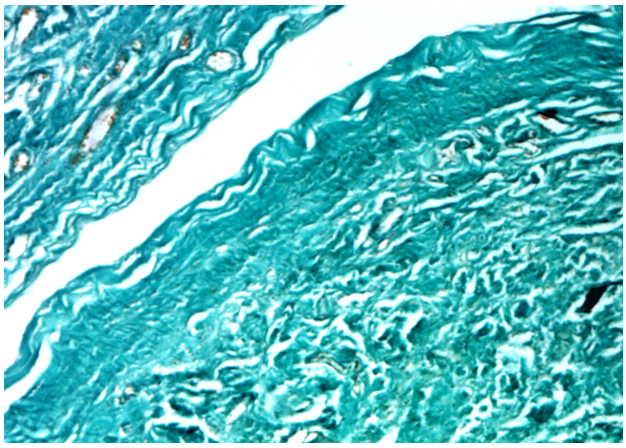

Figure 7 – ***Stromal fibrosis, after eight years of PD. Trichromic Szekely staining, ×40.***

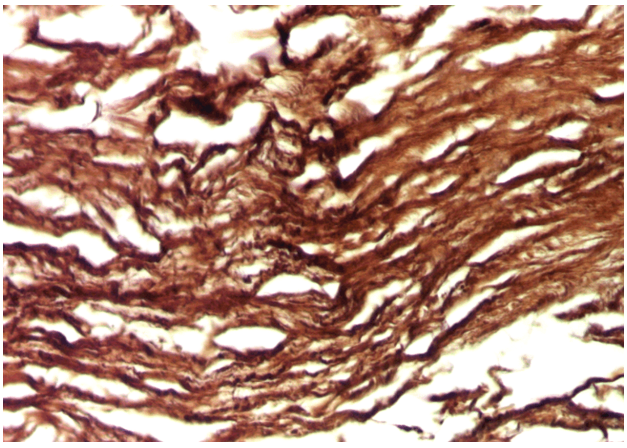

Figure 8 – ***Fibrous peritoneum with submesothelial elastic layer. Gordon–Sweet staining, ×200.***

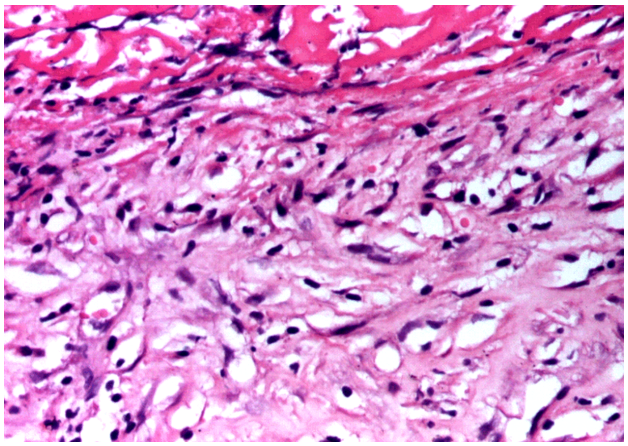

Figure 9 – ***Fibrous peritonitis, with apical location. HE staining, ×200.***

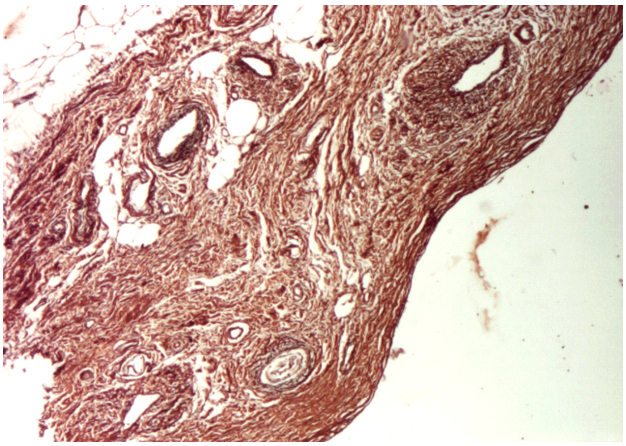

Figure 10 – ***Vascular wall thickening after five years of PD. Gordon–Sweet staining, ×40.***

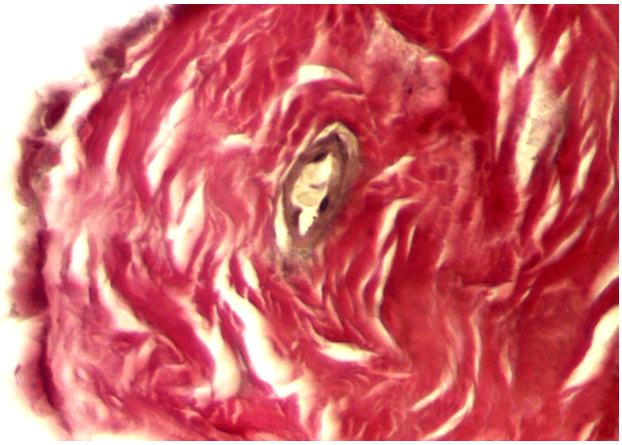

Figure 11 – ***Hyalinization of a capillary with total obstruction (14 years of PD). Van Gieson staining, ×400.***

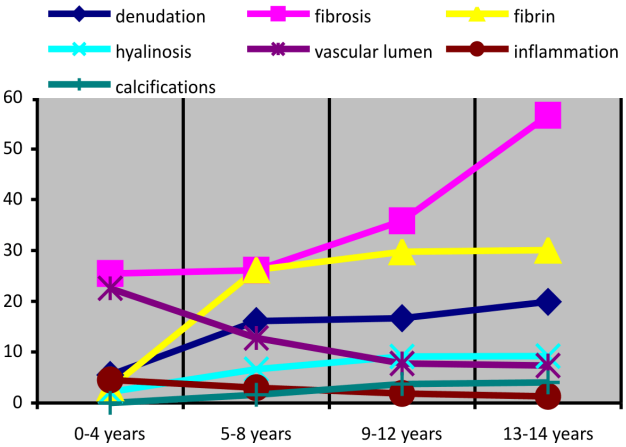

Figure 12 – ***Graphic expression of peritoneal morphological changes.***

Discussion

Tanned and hardbound peritoneal aspect was observed in patients with PD over five years (in which progressive fibrosis evaluated by stereology showed percentage volumes of 26.1%, 35.85% and 56.63%. Brown plates (of 1–2 cm) were found mainly in patients with peritoneal adhesions disease or with recurrent infectious peritonitis, which became surgical situations. The tanned aspect of the peritoneal surface and the presence of dispersed brown hardbound plates were previously observed in patients with PD [4], with starting fibrosis, confluence trend, hyperpigmentation all associated to extensive fibrosis in encapsulated peritoneal sclerosis. PD is deteriorating the functional morphology of the peritoneal serosa.

Stereological analysis performed in the present study emphasized progressive mesothelial exfoliation by percentage volumes from 5.49% (Group A) to 16.1% (Group B), 16.68% (Group C), and 19.88% in the patients with a 14 years PD (with no episode of microbial inflammatory peritonitis). During histological evaluation, reduplication of the mesothelial basement membrane was

assessed in 11 patients with PD over 60 months (a total of 10% and 61.11% in patients with PD from 5–14 years). In chronic PD patients was found that besides mesothelial denudation, mesothelial aquaporines' activity is lost, with decreased CA125 levels as marker of active mesothelial cells in these patients; in chronic PD the ultrafiltration ability of the peritoneal membrane is arrested [11].

Peritoneal inflammation in chronic PD induces a scar-forming healing process, which involves the submesothelial fibroblasts. Activation and proliferation of the peritoneal fibroblasts generates interstitial stromal fibrosis. The key mediator for the peritoneal fibrosis is TGFβ, which is produced by the inflammatory cells and inhibits the apoptosis of fibroblasts [12, 13]. An intermediate, myoid/fibroblastic, phenotype of the stromal cells is absent in normal and non-PD patients but it shows a maximal density (myofibroblastic conversion) beneath the mesothelial layer in chronic PD patients with peritoneal impairment [14–18]. In the presence of the inflammatory infiltrate, the stromal cells of the peritoneum are involved in progressive fibrosis, in modulating the inflammatory response, in tissue contraction, and in angiogenesis and vasculopathy [19]. Repeated episodes of peritonitis or hemoperitoneum may accelerate these processes, which, ultimately, lead to ultrafiltration failure [19]. Submesothelial myofibroblasts originating from mesothelial cells through epithelial–mesenchymal transition (EMT) and from resident fibroblasts are involved in inflammatory responses, extracellular matrix accumulation, and angiogenesis [18]. The mesenchymal phenotype of trans-differentiated mesothelial cells may retain a permanent mesenchymal phenotype as long as initiating stimuli persist, and contribute to PD-induced fibrosis and angiogenesis [19]. Emerging evidence point to the peritoneal microvasculature as the main factor responsible for increased solute transport and ultrafiltration failure although the pathophysiology of peritoneal fibrosis and angiogenesis remains elusive [19]. Experiments should find solutions to block, or reverse the EMT of mesothelial cells in order to prevent the fibrotic and angiogenetic mechanisms of ultrafiltration failure [19].

An autopsy study identified the correlation between the subendothelial hyalinization, the submesothelial fibrosis and the ultrafiltration decrease or failure, by severe impairment of the peritoneal membrane function after 3–4 years of PD [20]. Vascular subendothelial progressive hyaline sclerosis and stenosis up to occlusive lumen obliteration were identified in progressive percentage values from 2.22% to 6.63%, 9.16%, and 9.29% in our patient groups. At the same time, we noticed a reduction in permeability for the vascular lumen, updated to a complete obliteration and expressed in percentage volumes from 22.59% to 12.81%, 7.77%, and 7.37%. Our data are consistent with the observations of Devuyst *et al.* [9] and De Vriese *et al.* [21] who observed an increased vasculopathy incidence with PD duration and the degree of peritoneal fibrosis in patients with classic PD.

Osteopontin, which is also expressed in PD patients [22–24], actively participates to the dystrophic calcification of the peritoneum. In this study, calcifications mostly occurred in the walls of hyalinized vessels and were observed at 5–8 years of PD, with percentage volumes of 1.63%, 3.74%, and 4.03%. Perivascular inflammation recorded decreasing values at five years of PD from 4.55% to 2.98%, 1.87%, and 1.33%.

In order to limit or to delay the pathological changes of peritoneum and to extend the functionality of the peritoneal membrane, several approaches could be considered: (a) glucose replacement with other biocompatible osmotic agents; (b) protocol changes of the sterilization process and lactate buffer replacement with bicarbonate; (c) limitation of microbial inflammatory episodes in the peritoneum; (d) reduction of hypertonic dialyzing solution usage during infectious peritonitis episodes (to reduce peritoneal stress, to quicken mesothelial re-epithelialisation, and to limit progressive fibrosis, encapsulated peritoneal sclerosis, angiogenesis and ultrafiltration failure); (e) recombined human erythropoietin treatment to prevent [25] the dialysis fluid-induced apoptosis of mesothelial cells.

Conclusions

Histological and stereological data quantification illustrates structural and functional alteration of the dialysis membrane in long-term PD. These should be further evaluated with specific biomarkers in order to adequately assess the role of EMT and angiogenesis in chronic PD. Peritoneal dialysis is the therapeutic procedure in terminal stage chronic renal failure and may induce by itself or in association with peritoneal inflammatory infectious episodes, at 3–4 years of treatment, a cascade of self-maintained peritoneal structural changes, supported by the chronically stressed mesothelial cells. The recorded PD efficiency depends upon a reduced number of infectious peritonitis episodes.

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